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AMELIORATING EFFECT OF VITAMIN E AND VITAMIN C ON CADMIUM INDUCED HEPATOTOXICITY IN LABORATORY CHICKS

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ABSTRACT

Cadmium is a well-known human carcinogen and a potent hepatotoxin induce severe liver damage by altering several marker enzymes. Vitamin E and Vitamin C are effective antioxidants and free radical scavenger against metal toxicity. Therefore, present study has been design to investigate the potential protective effect of vitamin E and vitamin C separately and in combined form against hepatotoxicity induced by cadmium chloride in laboratory chicks. Investigation was carried out by monitoring the AST, ALT and ALP level in liver. The level of AST, ALT and ALP increased significantly ($p<0.005$) in cadmium treated chicks as compared to control group, vitamin E and vitamin C treated groups. Present results reveals that intoxication of cadmium chloride ($CdCl_2$) induce hepatotoxicity and disturb the liver functions. When cadmium chloride treated chicks administered with vitamin E and vitamin C separately, the level of protection reach up to control level. On other hand, co- administration of both vitamins (Vit E + Vit. C) in combined form lead to the most significant protection against cadmium toxicity in comparison to vitamin E and vitamin C separately. In conclusion, results demonstrated that intoxication of cadmium chloride ($CdCl_2$) induce hepatotoxicity and co-administration of antioxidant i.e. vitamin E and vitamin C separately or in combined form leads to significant decrease in AST, ALT and ALP values in liver and exhibit improvement in liver functions. However, combined form of both vitamins was observed most protective.

Key words: $CdCl_2$, Vitamin E, Vitamin C, AST, ALT and ALP

1. INTRODUCTION

Cadmium (Cd) as a toxic heavy metal has been distributed widely and uniformly with small amount throughout the earth's crust. Cd is not considered as essential element for living organisms, therefore its presence in organism tissues is considered as contamination (Rehman et al., 2012). This element is known as one of the most important environmental and industrial toxic agents and affects many target tissues such as appetite and pain centers, brain, heart and blood vessel, kidney and lungs (Bendeddouche et al., 2014). In people occupationally exposed to Cd, main route of entry into the body is Cd inhalation, but also the intake of Cd via digestive tract and skin contributes total exposure (Velickov et al., 2013). It is a toxicant that has a long biological half-life (15-20 years) and accumulates over time within the blood, kidneys, and liver (Michael and Jorge, 2004). Cd causes poisoning in various tissues of humans and animals (Stosh et al., 2000).

Evidence suggests that Cd exposure enhances intracellular reactive oxygen species production and lipid peroxidation, which may led to tissue damage (Kara et al., 2005). Liver and kidneys are important organs of metabolism, detoxification, storage, and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage (Brzoska et al., 2003). The greatest body accumulation of Cd occurs in the liver and kidney (Kang et al., 2016). Cd is toxic to several tissues, most notably causing hepatotoxicity as well as

nephrotoxicity upon exposure (Solaiman et al., 2001). It can induce biochemical changes in liver and kidney. In kidney it causes an increase in urea, uric acid, and creatinine (Maheswari and Venkatnarayanan, 2013). In kidney it causes shrinkage or regeneration of cells of the renal tubules, pyknoric nuclei, and haemorage (El-Refaiy and Eissa, 2013). It also causes oxidative stress related renal dysfunction (Renugadevi and Prabu, 2010). Histologically, treatment with Cd causes severe damage, including fatty changes, necrosis, pyknotic nuclei, karayolysis, and proliferation of kupffer cells (Albasha and Azab, 2014). Hepatic disorders were noticed in rats as manifested by increase of liver enzymes (Jevtovic-Stoimenov et al., 2005).

Vitamins are essential to maintain normal metabolic processes and homeostasis within the body. Vitamin C (Vit C) and vitamin E (Vit E) are low molecular mass antioxidants that scavenge or quench free radicals (Janisch et al., 2005). These findings suggest potential role of antioxidants to ameliorate cadmium toxicity. Both Vit C and Vit E alleviate oxidative stress associated with a variety of pollutants. Vit C and Vit E reduced lipid peroxidation and oxidative stress result from arsenic (Kannan and Flora, 2004), Ozone (Sienra-Monge et al., 2004) and cadmium toxicity (Grosicki, 2004).Vitamin C and vitamin E are recognized as essential nutrients for all species of animals. In other words, these vitamins have been shown to have protective effect against metal induced toxicity (Rao and Sharma, 2001; Jiraungkoorskul et al., 2007) Therefore, the present study was designed to evaluate the effect of vitamin E and vitamin C separately and in combined form against cadmium chloride induced liver hepatotoxicity in chicks.

2. MATERIALS AND METHODS

The experiment was carried out on domestic chicks-Croiler Chabro (*Gallus gallus domesticus*). Newly hatched chicks were purchased from the Uttarakhand Village Poultry Project (State Govt. Poultry Farm), Bin, Pithoragarh (Uttarakhand). Selected chicks were maintained and acclimatized according to the laboratory conditions and housed in battery cages under laboratory conditions at existing room temperature and relative humidity. They were fed on commercial food (Starter, Grower and Finisher) purchased from the local market (Godrej company) and tap water *ad libitum.* Healthy male and female chicks (approximately 2-3 weeks old, body weight 100±20 gm) were used in present study. The animals were assigned to five groups (A, B, C, D and E) each of which contains six chicks. The chicks were treated daily for 1 month as follows:

Group A: Treated as control and administered with saline and commercial food supplied by local market.

Group B: Treated with $CdCl_2$ (5 mg/100 gm body weight) by gavage on each alternate day for 30 days.

Group C: Administered with cadmium chloride as group B and also supplemented with vitamin C (20mg/100 gm body weight) by gavage on each alternate day for 30 days.

Group D: Administered with cadmium chloride as group B and also supplemented with vitamin E (0.5IU /100 gm) intramuscularly on each alternate day for 30 days.

Group E: Administered with cadmium chloride and also supplemented with vitamin E and vitamin C in combined form (Vit E + Vit C) on each alternate day for 30 days.

Vitamin C, Vitamin E and $CdCl_2$ were procured from Sigma Chemicals, USA. Blood samples were collected from the wing vein using 3.0 ml disposable syringe and directly transferred into a labeled test tube. Plasma was prepared by centrifugation and stored at 0ºC for further analysis. The plasma AST, ALT and ALP enzymatic activity were determined using commercial kits supplied by Span Diagnostic Limited (Ahmadabad, India).

Statistical analysis: All plasma AST, ALT and ALP values were expressed as mean ± SE. Parameters of all treatments were compared using Student's "t" test. Data were subjected to one way ANOVA for calculating the significance difference between the treated and control group. The level of significance were reported at $p<0.05$.

3. RESULT

Enzymes markers of liver function viz. AST, ALT and ALP offer reliable information on status of liver function (Table- 1). Present results reveals that activity of AST, ALT and ALP significantly increased ($p<0.05$) in cadmium treated chicks relative to that obtained from the control group. However, when cadmium chloride treated chicks were co-administered with vitamin E and vitamin C separately, the activities of AST, ALT and ALP being significantly ($p<0.05$) decreased indicate protection offered by these antioxidants. Observations indicate that vitamin E is seems to more protective in comparison to vitamin C.

Further, co-administration of both vitamin E and vitamin C combinedly (Vit E + Vit C) in Cd- treated chicks (group E) exhibited more protection than groups supplemented with either of the vitamins E and vitamin C separately. The chicks supplemented with antioxidants i.e. vitamin E and vitamin C simultaneously shows protection against cadmium toxicity and improve the liver functions. Supplementation of these antioxidants reduces the accumulation of cadmium in liver by different channelized manner, and restore the liver functions nearest to control level. Although, combined administration of vitamin E and vitamin C is seems to more protective in comparison to separate supplementation.

Table 1. Protective effect of vitamin E and vitamin C saperately and in combined form on AST, ALT and ALP in cadmium chloride treated chicks.

Grroup	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Level of Significance
A	Control	107.41 ± 2.65	12.65 ± 2.53	26.72± 1.02	**
B	Cadmium	131.12 ± 1.96	24.23 ±0 .33	57.05 ± 1.46	**
C	Cadmium +Vitamin C	116.36 ± 1.53	14.42 ± 0.55	29.85 ± 2.00	**
D	Cadmium + Vitamin E	113.13 ± 2.79	14.72 ± 2.02	30.07 ± .96	**
E	Cadmium + Vit C + Vit E	108.77 ± 2.82	13.00 ± 2 .00	28.00 ± .67	**

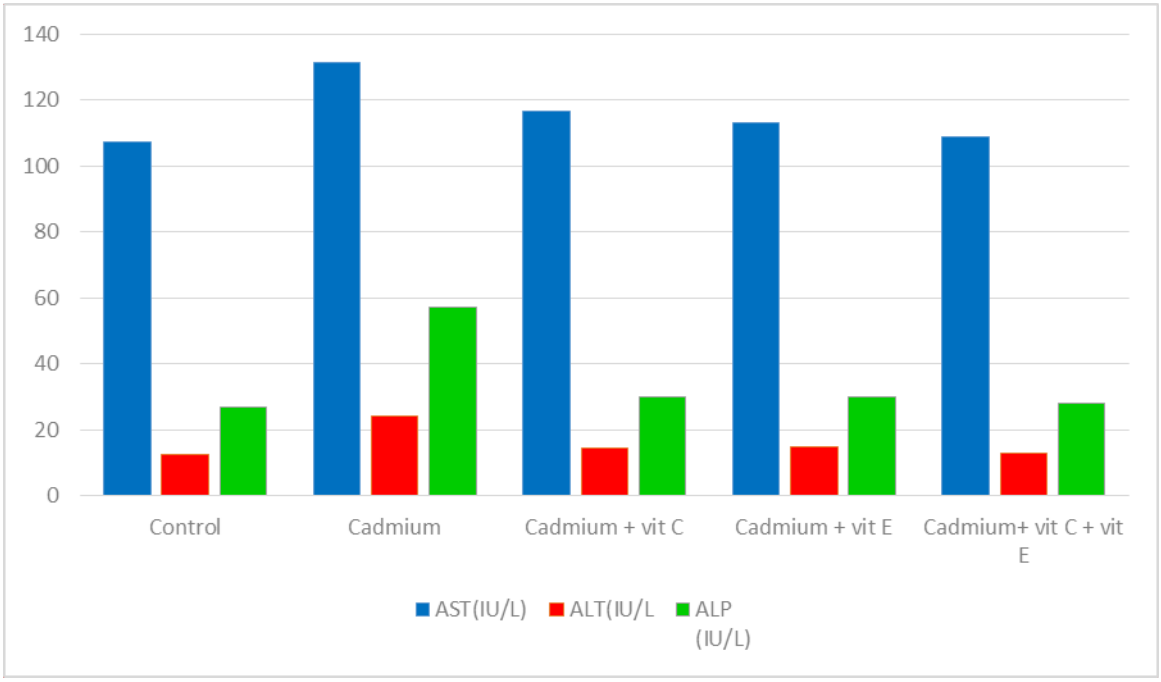

Fig 1. Protective effect of vitamin E and vitamin C separately and in combined form on AST, ALT and ALP in cadmium treated chicks.

4. DISCUSSION

Cadmium is a toxic metal that is widely used in different industries has a long biological half-life (15-20 years) and non-essential for human population and highly toxic to animals and plants (Zhuang et al., 2016). Cadmium causes loss in the ability of the plasma membrane to act as a barrier, leading to the loss of catalytic enzymes and substrates from intracellular stores (Alvarez and Storey, 1984). Traditionally evaluation of liver function is achieved by assessing the activities of hepatic enzymes in plasma such as AST, ALT and ALP. The change in enzyme activities in plasma is reflecting the amount of enzymes that leak out from the membrane of damaged hepatocytes, rather than the change in real liver function. In general, the toxic effect of $CdCl_2$ on the activities of several enzymes and generation of free radicals may be due to a large number of cellular processes including its replacement of zinc in many vital enzymatic reactions. Also, Cd replaces calcium in calcium binding proteins causing disruption or cessation of activity which can lead to oxidative stress.

The current study demonstrated a significant increase ($p<0.05$) in AST, ALT and ALP in plasma of chicks treated with cadmium chloride. The increase in liver enzymes can be attributed to the increase in the level of lipid peroxidation. The increase in plasma AST, ALT and ALP activities (Table-1) are in agreement with previous findings that Cd caused alterations in liver transaminases of rats (Rana et al., 1996). Similar observations were reported in many experimental investigations on animals exposed to Cd (Albasha and Azab, 2014). The increased levels of studied enzymes in Cd-induced chicks indicate an increased permeability and damage and/or necrosis of hepatocytes (Ojo et al.,2014), which increase the release of these enzymes in the bloodstream (Jaramillo-Jurez et al., 2003). Similar result demonstrated by authors who found that increased activity of enzymes AST and ALT in guinea pigs (Hassan et al., 2012) and in rats (Gaurav et al., 2011).The increase in concentration of AST and ALT in blood plasma indicates hepatotoxic effect of $CdCl_2$.

Present results are in agreement of previous studies that sublethal concentration of Cd caused significant increases in AST, ALT and ALP activity of laboratory chicks. A significant increase ($P<0.05$) in AST, ALT and ALP activity was recorded in cadmium chloride treated groups. However when these groups was supplemented with antioxidants i.e. vitamin E and vitamin C, the level of protection in liver function was observed. Vitamin E offer more protection against cadmium toxicity in comparison to vitamin C. On other hand, co-administration of both vitamin E and vitamin C combinedly (Vit E + Vit C) in Cd- treated chicks exhibited more protection than groups supplemented with either of the vitamins E and vitamin C separately.

The activity of AST, ALT and ALP enzymes in blood may also be used as a stress indicator. The significant changes in activities of these enzymes in blood plasma indicate tissue impairment caused by stress (James et al., 1992; Svoboda, 2001). In the present study there were significant changes in AST, ALT and ALP activities in plasma of chicken exposed to cadmium chloride compared to control group. The increase in concentration of AST, ALT and ALP in blood plasma indicates impairment of liver. In addition, the increase in plasma AST, ALT and ALP may be attributed to the hepatocellular damage or cellular degradation by cadmium chloride in liver.

Vitamin E due to its solubility in lipids, plays an important role in protecting lipid-rich structure like liver from free radicals by chain breaking mechanism and an effective inhibitor of autocatalytic process of lipid peroxidation (Sodhi et al., 2008). The protective action of ascorbic acid on heavy metal toxicity is well documented via its free radical scavenging mechanism and detoxification effect (Suzuki, 1990). In the current study, co-administration of both vitamin C and vitamin E to Cd treated chicks exhibited more protection than the groups supplemented with either of the vitamins separately. Both vitamins restore liver functions from Cd induced toxic damage via chain breaking mechanism or their synergetic antioxidant activities.

The interaction among the two vitamins may maintain them in their reduced form to rebuild the endogenous antioxidant balances that could have been impaired by elevated oxidative stress following Cd administration. In addition, these findings are in good agreement with those obtained by other studies which postulated the beneficial role of vitamin E and vitamin C on histological and enzymatic changes of rats (Ben Amara et al., 2010). Therefore, the supplementation of vitamin E or vitamin C help in restoration of liver function from cadmium toxicity as indicated by the AST, ALT and ALP values.

5. CONCLUSION

It may be concluded from the present study that, vitamin C or vitamin E may be useful as a prophylactic agent for cadmium poisoning. The combined supplementation with vitamin C and vitamin E highly protected chicks from cadmium toxicity. It appears that vitamin C and vitamin E act synergistically in reducing cadmium toxicity.

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